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Cytotoxic Activity of 5-Benzoylimidazole and Related Compounds against Human Oral Tumor Cell Lines

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Abstract. *A total of 24 benzoylimidazoles and structurally-related compounds were investigated for their cytotoxic activity against oral tumor cells and normal gingival fibroblast. Compound* **23** *(5-(2-hydroxylbenzoyl)-2-phenylimidazole) showed the highest cytotoxic activity against both human oral tumor cell lines (human squamous cell carcinoma HSC-2, human salivary gland tumor HSG) and normal human gingival fibroblast (HGF). Compounds* **7** *(2-(2-hydroxybenzoyl)benz imidazo[2,1-b]thiazole),* **14** *(1,3-diethyl-5-(2-hydroxybenzoyl)-4-imidazoline-2-thione) and* **18** *(5-(2-hydroxy-4-methoxybenzoyl)-3-methyl-2-methylimino-4-thiazoline) showed slightly lower cytotoxic activity, but higher tumor-specific cytotoxic action. The cytotoxic activity of compound* **23** *was significantly reduced by $CuCl_2$, but not by $CoCl_2$, $FeCl_3$, or by antioxidants (N-acetyl-L-cysteine, sodium ascorbate, catalase). Compound* **23** *did not show any detectable oxidation potential (determined by NO monitor). Agarose gel electrophoresis demonstrated that compound* **23** *induced DNA fragmentation in human promyelocytic leukemia cells HL-60, but not in HSG cells. These data suggested that the response to compound* **23** *might be different from cell to cell.*

We have recently summarized the efficacy of polyphenols in preventing oral diseases (1). Our strategy include the following three steps: (i) screening of various natural and synthetic compounds, (ii) elucidation of action mechanism and, (iii) interaction with the oral environment. We have recently found that tannins, such as macrocyclic hydrolyzable tannins, epigallocatechin gallate (EGCG) (a major component of green tea), gallic acid (a component unit of tannin) (2-4) and isoprenylated flavonoids (5, 6) induced apoptotic cell death, characterized by DNA fragmentation (identified by TUNEL method and agarose gel electrophoresis) and caspase activation (identified by M30 monovlonal antibody), in human oral tumor cells. Lignins, which had little cytotoxic activity, synergistically stimulated the cytotoxic activity of sodium ascorbate (7). We have recently reported that newly synthesized benzoxepin/benzothiepin induced tumor-specific cytotoxicity and internucleosomal DNA fragmentation, a biochemical hallmark of apoptosis, in oral tumor cell lines (8). These findings prompted us to initiate the structure-activity relationship of various synthetic compounds, for their clinical application.

Imidazol(ine) derivatives inhibit the acetylcoline-induced secretion of catecholamines in adrenal chromaffin cells (9) by blocking nicotinic acetylcholine receptors (10). These drugs were previously reported to act as α_1-adenoceptor antagonists. Also, imidazol(ine) derivatives have been shown to be neuroprotective in brain injuries of necrotic (11) and apoptotic neuronal cell death (12). Previously, the neuroprotective effects of imidazolines against N-methyl-D aspartate (NMDA)-induced neurotoxicity in cultured cerebellar granule cells (13,14) have been reported. These imidazol(ine) derivatives possibly show the broad spectrum of biological effects on primary or permanent cells apart from the cells described above. We report here the relative potency of 5-benzoylimidazole and related compounds (15) to induce cytotoxicity against human oral squamous cell carcinoma cells HSC-2 and human salivary gland tumor cells HSG, in comparison with human normal gingival fibroblast HGF and human promyelocytic leukemia HL-60 cells.

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Key Words: 5-Benzoylimidazole, cytotoxic activity, oral tumor cells, DNA fragmentation.

Materials and Methods

Materials. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM), RPMI1640 medium (Gibco BRL, Gland Island, NY, USA); fetal bovine serum (FBS)(JRH Biosic, Lenexa, KS, USA); $CoCl_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, dimethyl sulfoxide (DMSO)(Wako Pure Chem. Ind. Ltd., Osaka, Japan), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium

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bromide (MTT), catalase, *N*-acetyl-L-cysteine (NAC) (Sigma Chem. Ind. St. Louis, MO, USA); RNase A, proteinase K (Boehringer Mannheim, Germany).

Synthesis of 3-azolylthio-4H-1-benzopyran-4-one and 2-benzoylimidazo [2,1-b]thiazole (general procedure A). A mixture of 3-iodochromone (136 mg, 0.5 mmol), mercaptoazoles (0.5 mmol) and K_2CO_3 (276 mg, 2 mmol) in DMF (5 mL) was stirred for 1-4 hours at room temperature. After removal of the K_2CO_3, the reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane-AcOEt).

Synthesis of 3-(1H-benzimidazol-2-ylthio)-4H-1-benzopyran-4-one (1) and 2-(2-hydroxybenzoyl)benzimidazo[2,1-b]thiazole (7). According to the general procedure A, 3-iodochromone (136 mg, 0.5 mmol) and 2-mercaptobenzimidazole (75 mg, 0.5 mmol) were treated with K_2CO_3 for 1 hour to give **1** (41 mg, 28%) and **7** (88 mg, 60%), respectively.

Synthesis of 3-(1H-benzmidazol-2-ylthio)-6-methoxy-4H-1-benzopyran-4-one (2). According to the general procedure A, 3-iodo-6-methoxychromone (151 mg, 0.5 mmol) and 2-mercaptobenzimidazole (75 mg, 0.5 mmol) were treated with K_2CO_3 for 4 h to give 2 (139 mg, 86%).

Synthesis of 3-(1H-imidazol-2-ylthio)-4H-1-benzopyran-4-one (3) and 2-(2-hydroxybenzoyl)imidazo[2,1-b]thiazole (10). According to the general procedure A, 3-iodochromone (136 mg, 0.5 mmol) and 2-mercaptoimidazole (50 mg, 0.5 mmol) were treated with K_2CO_3 for 1 hour to give **3** (22 mg, 18%) and **10** (76 mg, 62%), respectively.

Synthesis of 3-(1-methyl-1H-imidazol-2-ylthio)-4H-1-benzopyran-4-one (4). According to the general procedure A, 3-iodochromone (136mg, 0.5 mmol) and 2-mercapto-1-methylimidazole (57 mg, 0.5 mmol) were treated with K_2CO_3 for 1 hour to give **4** (114 mg, 88%).

Synthesis of 3-(1H-1,2,4-triazol-3-ylthio)-4H-1-benzopyran-4-one (5). According to the general procedure A, 3-iodochromone (136 mg, 0.5 mmol) and 3-mercapto-1,2,4-triazole (51 mg, 0.5 mmol) were treated with K_2CO_3 for 2 hours to give **5** (88 mg, 72%).

Synthesis of 3-(4-methyl-4H-1,2,4-triazol-3-ylthio)-4H-1-benzopyran-4-one (6). According to the general procedure A, 3-iodochromone (136 mg, 0.5 mmol) and 3-mercapto-4-methyl-1,2,4-triazole (58 mg, 0.5 mmol) were treated with K_2CO_3 for 2 hours to give **6** (91 mg, 70%).

Synthesis of 2-(2-hydroxybenzoyl)-7-methoxybenzimidazo[2,1-b]thiazole (8). According to the general procedure A, 3-iodochromone (136 mg, 0.5 mmol) and 2-mercapto-5-methoxybenzimidazole (90 mg, 0.5 mmol) were treated with K_2CO_3 for 1 hour to give **8** (73 mg, 45%).

Synthesis of 5H-benzimidazo[2', 1':2, 3]thiazolo[4,5-b]benzopyran-5-one (9). To a stirred solution of **7** (84 mg, 0.3 mmol) and DBU (182 mg, 1.2 mmol) in CH_2Cl_2 (3 mL), a solution of iodine (84 mg, 0.33 mmol) in CH_2Cl_2 (2 mL) was drop-wise added over a 20 minutes period at 0°C. After being stirred for 10 minutes, the reaction was quenched at the same temperature by adding saturated aqueous $Na_2S_2O_3$ (2 mL). The mixture was extracted with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give 9 (81 mg, 92 %).

Synthesis of 5-benzoyl-4-imidazoline-2-thione and 5-benzoyl-2-imino-4-thiazoline (general procedure B). A mixture of 3-iodochromone (136 mg, 0.5 mmol), thiourea (2 mmol), Bu_4NCl (139 mg, 0.5 mmol) and K_2CO_3 (690 mg, 5 mmol) in benzene (15 mL) was refluxed for 10-18 hours. After removal of K_2CO_3, the reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane-AcOEt).

Synthesis of 5-(2-hydroxylbenzoyl)-1,3-dimethyl-4-imidazoline-2-thione (11) and 5-(2-hydroxybenzoyl)-3-methyl-2-methylimino-4-thiazoline (16). According to the general procedure B, 3-iodochromone (136 mg, 0.5 mmol) and 1,3-dimethyl-2-thiourea (208 mg, 2 mmol) were treated with K_2CO_3 for 18 hours to give **11** (78 mg, 63%) and **16** (33 mg, 27%), respectively.

Synthesis of 5-(2-hydroxyl-5-methoxylbenzoyl)-1,3-dimethyl-4-imidazoline-2-thione (12) and 5-(2-hydroxy-5-methoxybenzoyl)-3-methyl-2-methylimino-4-thiazoline (17). According to the general procedure B, 3-iodo-6-methoxylchromone (151 mg, 0.5 mmol) and 1,3-dimethyl-2-thiourea (208 mg, 2 mmol) were treated with K_2CO_3 for 15 hours to give **12** (85 mg, 61%) and **17** (44 mg, 32%), respectively.

Synthesis of 5-(2-hydroxyl-4-methoxylbenzoyl)-1,3-dimethyl-4-imidazoline-2-thione (13) and 5-(2-hydroxy-4-methoxybenzoyl)-3-methyl-2-methylimino-4-thiazoline (18). According to the general procedure B, 3-iodo-7-methoxylchromone (151 mg, 0.5 mmol) and 1,3-dimethyl-2-thiourea (208 mg, 2 mmol) were treated with K_2CO_3 for 10 hours to give **13** (174 mg, 80%) and **18** (20 mg, 14%), respectively.

Synthesis of 1,3-diethyl-5-(2-hydroxybenzoyl)-4-imidazoline-2-thione (14). According to the general procedure B, 3-iodochromone (136 mg, 0.5 mmol) and 1,3-diethyl-2-thiourea (264 mg, 2 mmol) were treated with K_2CO_3 for 14 hours to give 14 (124 mg, 90%).

Synthesis of 5-(2-hydroxybenzoyl)-1,3-diphenyl-4-imidazoline-2-thione (15). According to the general procedure B, 3-iodochromone (136 mg, 0.5 mmol) and 1,3-diphenyl-2-thiourea (456 mg, 2 mmol) were treated with K_2CO_3 for 15 hours to give **15** (169 mg, 91%).

Synthesis of 4,5,6,7-tetrahydro-2-(2-hydroxybenzoyl)pyrimido[2,1-b]thiazole (19). According to the general procedure B, 3-iodochromone (136 mg, 0.5 mmol) and 3,4,5,6-tetrahydro-2-pyrimidinethiol (232 mg, 2 mmol) were treated with K_2CO_3 in MeCN at rt for 6 hours to give 19 (129 mg, 99%).

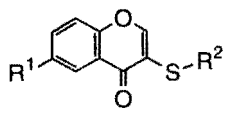
Synthesis of 5-benzoylimidazole (general procedure C). A mixture of 3-iodochromone (136 mg, 0.5 mmol), amidines (2 mmol), and K_2CO_3 (690 mg, 5 mmol) in DMF (10 mL) was stirred for 1-5 h at rt. After removal of K_2CO_3, the reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane-AcOEt).

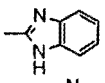
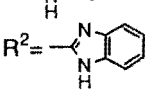
Synthesis of 5-(2-hydroxylbenzoyl)-2-methylimidazole (20). According to the general procedure C, 3-iodochromone (136 mg, 0.5 mmol) and acetamidine hydrochloride (189 mg, 2 mmol) were treated with K_2CO_3 for 1 hour to give 20 (93 mg, 92%).

Synthesis of 5-(2-hydroxy-5-methoxybenzoyl)-2-methylimidazole (21). According to the general procedure C, 3-iodo-6-methoxychromone (151 mg, 0.5 mmol) and acetamidine hydrochloride (189 mg, 2 mmol) were treated with K_2CO_3 for 2 hours to give **21** (111 mg, 96%).

Synthesis of 5-(2-hydroxy-4-methoxybenzoyl)-2-methylimidazole (22). According to the general procedure C, 3-iodo-7-methoxychromone (151 mg, 0.5 mmol) and acetamidine hydrochloride (189 mg, 2 mmol) were treated with K_2CO_3 for 3 hours to give **22** (111 mg, 96%).

3-azolylthio-4*H*-1-benzopyran-4-one

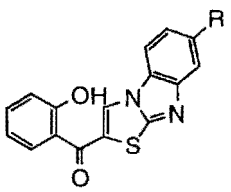


1: R^1=H, R^2= 
2: R^1=OMe, R^2= 
3: R^1=H, R^2= 
4: R^1=H, R^2= 
5: R^1=H, R^2= 
6: R^1=H, R^2= 

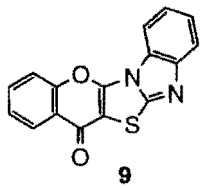
or

1: R^1=H, R^2=1*H*-benzimidazol-2-yl
2: R^1=OMe, R^2=1*H*-benzimidazol-2-yl
3: R^1=H, R^2=1*H*-imidazol-2-yl
4: R^1=H, R^2=1-methyl-1*H*-imidazol-2-yl
5: R^1=H, R^2=1*H*-1,2,4-triazol-3-yl
6: R^1=H, R^2=4-methyl-4*H*-1,2,4-triazol-3-yl

2-benzoylimidazo[2,1-*b*]thiazole



7: R=H
8: R=OMe



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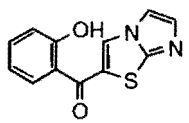

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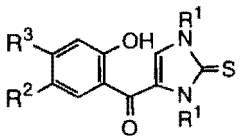
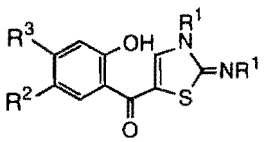
5-benzoyl-4-imidazoline-2-thione



11: R^1=Me, R^2=R^3=H
12: R^1=Me, R^2=OMe, R^3=H
13: R^1=Me, R^2=H, R^3=OMe
14: R^1=Et, R^2=R^3=H
15: R^1=Ph, R^2=R^3=H

5-benzoyl-2-imino-4-thiazoline



16: R^1=Me, R^2=R^3=H
17: R^1=Me, R^2=OMe, R^3=H
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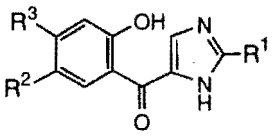
19: R^1=-$(CH_2)_3$-, R^2=R^3=H

5-benzoylimidazole



20: R^1=Me, R^2=R^3=H
21: R^1=Me, R^2=OMe, R^3=H
22: R^1=Me, R^2=H, R^3=OMe
23: R^1=Ph, R^2=R^3=H
24: R^1=SMe, R^2=R^3=H

Figure 1. *Structure of 3-azolylthio-4H-1-benzopyran-4-one (**1-6**), 2-benzoylimidazo[2,1-b] thiazole (**7-10**), 5-benzoyl-4-imidazoline-2-thione (**11-15**), 5-benzoyl-2-imino-4-thiazoline (**16-19**) and 5-benzoylimidazole (**20-24**).*

Table I. *Cytotoxic activity and tumor-specificity of 5-benzoylimidazole and related compounds.*

Compound	Cytotoxic activity (CC_{50}: μg/mL)				Tumor specificity	
	HSC-2 (A)	HSG	HL-60 (B)	HGF (C)	C/A	C/B
3-azolylthio-4H-1-benzopyran-4-one						
1	76	50				
2	15	76				
3	174	221				
4	>400	>400				
5	225	218				
6	296	330				
2-benzoylimidazo[2,1-b]thiazole						
7	52	44	17	183	3.5	10.8
8	>400	231				
9	164	264				
10	152	244				
5-benzoyl-4-imidazoline-2-thione						
11	104	128				
12	75	145				
13	50	136				
14	59	35	27	141	2.4	5.2
15	>400	310				
5-benzoyl-2-imino-4-thiazoline						
16	104	87				
17	144	85				
18	149	13	10	>400	2.7	>40
19	>400	295				
5-benzoylimidazole						
20	185	183				
21	191	247				
22	130	162				
23	27	18	4.2	24	0.89	5.8
24	77	80				

Near confluent cells were incubated for 24 hours with various concentrations of the indicated compounds. The relative viable cell number was then determined by the MTT method (HSC-2, HSG, HGF) or by trypan blue exclusion (HL-60). Control A_{540} of HSC-2, HSG and HGF cells were 1.376, 0.594 and 0.489, respectively. Each value represents the mean from 2 independent experiments which were performed in duplicate.

Table II. *Effect of antioxidants and metals on the cytotoxic activity of compound 23.*

Addition	Cytotoxic activity of **23** against HSG cells (CC_{50}: μg/mL)
None (control)	32
+ 4 mM NAC	25
+ 0.25 mM sodium ascorbate	28
+ 3000 unit/ml catalase	30
+ 0.2 mM $CoCl_2$	35
+ 0.2 mM $FeCl_3$	28
+ 0.2 mM $CuCl_2$	5.4

Near confluent HSG cells were incubated for 24 hours with various concentrations of compound **23** in the absence (control) or presence of the indicated concentrations of antioxidants or metals. The relative viable cell number was then determined by the MTT method to calculate the CC_{50} value. Control A_{540} of HSG cells was 1.140. Each value represents the mean from 2 determinations.

Synthesis of 5-(2-hydroxylbenzoyl)-2-phenylimidazole (23). According to the general procedure C, 3-iodochromone (136 mg, 0.5 mmol) and benzamidine hydrochloride (313 mg, 2 mmol) were treated with K_2CO_3 for 0.5 hours to give **23** (128 mg, 97%).

Synthesis of 5-(2-hydroxylbenzoyl)-2-methylthioimidazole (24). According to the general procedure C, 3-iodochromone (136 mg, 0.5 mmol) and methylisothiourea sulfate (556 mg, 2 mmol) were treated with K_2CO_3 for 5 hours to give **24** (98 mg, 84%).

Cell culture. Human oral squamous cell carcinoma (HSC-2) cells human salivary gland tumor (HSG) cells and human gingival fibroblast (HGF) (7-9th passage) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. Human promyelocytic leukemia HL-60 cells were cultured in RPMI1640 medium supplemented with 10% FBS (16).

Assay for cytotoxic activity. Near confluent HSC-2, HSG and HGF cells grown in 96-microwell plates (Falcon, flat bottom, treated polystyrene, Becton Dickenson) were incubated for 24 hours with various concentrations of samples. The cells were washed with phosphate-buffered saline and incubated for 4 hours with fresh culture medium containing 0.2 mg/mL MTT. After removing the medium, the cells were lysed with 100 μl DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate with Labsystem Multiskan^R (Biochromatic) with Star/DOT Matrix Printer JL-10. The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve (7).

Assay for redox potential. Compounds **7, 14, 18** or **23** (final: 50 μg/mL) were added to 10 mL of DMEM medium supplemented with 10% FBS and mixed by constant stirring with a magnet stirrer. The redox potential was measured at the indicated time points thereafter with NO

monitor (Inter Medical Co., Ltd., Nagoya, Japan) and expressed as the difference (ΔmV) from the initial value (17).

Assay for DNA fragmentation. The cells were pelleted, lysed and digested with RNase A and proteinase K. DNA was isolated and assayed for DNA fragmentation by 2% agarose gel electrophoresis (16). DNA from apoptotic HL-60 cells induced by UV irradiation (18) was run in parallel as a positive control.

Results and Discussion

Among 24 compounds, compound **23** (5-(2-hydroxylbenzoyl)-2-phenylimidazole) showed the highest cytotoxic activity against two human oral tumor cell lines (HSC-2, HSG) (CC_{50}=**27** and 18 mg/mL, respectively) (Table I). Compounds **7** (2-(2-hydroxybenzoyl)benzimidazo[2,1-*b*] thiazole) (CC_{50}=52, 42 μg/mL), **14** (1,3-diethyl-5-(2-hydroxybenzoyl)-4-imidazoline-2-thione) (CC_{50}=59, 35 μg/mL) and **18** (5-(2-hydroxy-4-methoxybenzoyl)-3-methyl-2-methylimino-4-thiazoline) (CC_{50}=149, 13 μg/mL) showed slightly lower cytotoxic activity.

Tumor specificity. We found that normal fibroblasts (HGF cells) were relatively resistant to compounds **7, 14** and **18** (tumor specific ratio (C/A)=3.5, 2.4 and 2.7, respectively, but highly sensitive to compound **23** (C/A=0.89) (Table I). On the other hand, human promyelocytic leukemic HL-60 cells were very sensitive to all 4 compounds (C/B=11, 5.2, >40.0 and 5.8, respectively) (Table I).

Effect of antioxidants and metals. It was of interest to test the possibility that compound **23** might induce cytotoxicity by its prooxidant action. Table II shows that the cytotoxic activity of compound **23** was not significanty affected by the optimum concentrations of antioxidants, such as NAC (4 mM), sodium ascorbate (0.25 mM) or catalase (3,000 unit/mL) (which decomposes hydrogen peroxide). These data reduced the possibility of prooxidant action of compound **23**. Among 3 metals investigated, only $CuCl_2$ significantly (5-fold) enhanced the cytotoxic activity of compound **23**, whereas $CoCl_2$ and $FeCl_3$ were inactive (Table II).

Induction of DNA fragmentation. Agarose gel electrophoresis showed that compound **23** dose-dependently induced DNA fragmentation in HL-60 cells, but not in HSG cells (Figure 2). However, there was a narrow range of optimal concentration, and higher and lower concentrations failed to induce DNA fragmentation. These data suggested that the response to compound **23** might be different from cell to cell.

Among **24** compounds, we selected four cytotoxic compounds **7, 14, 18** and **23**. Compounds **7, 14, 18** selectively killed the tumor cells. The most cytotoxic compound **23** did not show such tumor-specific action, but induced DNA fragmentation in HL-60 cells. We found that the cytotoxic activity of compound **23** was not reduced by antioxidants, nor by $CoCl_2$. The lack of $CoCl_2$ sensitivity can be explained by the absence of the diol structure in the molecule (19). We also found that $CuCl_2$ enhanced the cytotoxic activity of compound **23** by a yet unknown mechanism. We have recently found that $CuCl_2$ slightly enhanced the cytotoxic activity of 4-chloro-3,4-dihydro-2-(2-oxo-2-phenylethyl)-1-benzothiepin-5-one and 2,3-dihydro-2-(2-oxopropyl)-2-phenyl-1-benzoxepin (8). There might be common stimulation mechanisms between these compounds. Further studies of the mechanism of their cytotoxic action are under way in our laboratory.

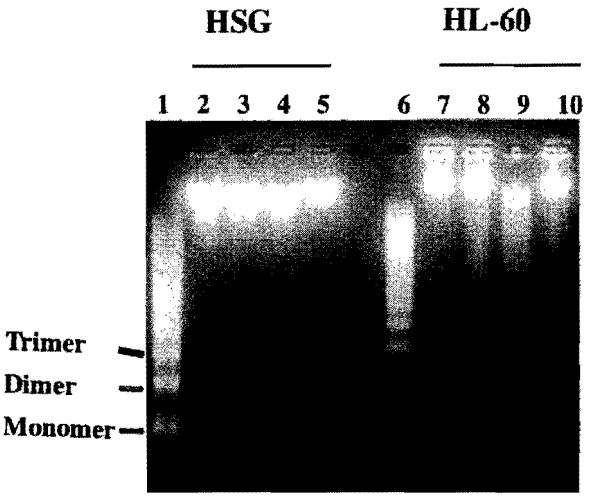


Figure 2. *Induction of DNA fragmentation by compound* **23**. *(A) Near confluent HSG cells (lane 2-5) and HL-60 cells ($1x10^6$/mL) (lanes 7-10) (B) were incubated for 6 hours with 0 (control) (lanes 2, 7), 20 (lanes 3, 8) 40 (lanes 4, 9) or 80 (lanes 5, 10) μg/mL of compound* **23**. *DNA was then extracted and subjected to 2% agarose gel electrophoresis. Lanes 1 and 6 are the DNA from apoptotic HL-60 cells induced by UV irradiation (18).*

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References

1 Sakagami H, Oi T and Satoh K: Prevention of oral diseases by polyphenols (Review). In Vivo *13:* 155-171, 1999.

2 Ishino A, Kusama K, Watanabe S and Sakagami H: Inhibition of epigallocatechin gallate-induced apoptosis by $CoCl_2$ in human oral tumor cell lines. Anticancer Res *19:* 5197-5202, 1999.

3 Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Ito H, Hatano T and Yoshida T: Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines - A possible mechanism. Phytomedicine *7:* 39-47, 2000.

4 Jiang Y, Kusama K, Satoh K, Takayama F, Watanabe S and Sakagami H: Induction of cell death by chlorogenic acid in human oral tumor cell lines. Phytomedicine *7:* 483-491, 2000.

5 Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Fukai T and Nomura T: Induction of apoptosis by flavones, flavonols (3-hydroxylflavones) and isoprenoid-substituted flavonoids in human oral tumor cell lines. Anticancer Res *20:* 271-278, 2000.

6 Fukai T, Sakagami H, Toguchi M, Takayama F, Iwakura I, Atsumi T, Ueha T, Nakashima H and Nomura T: Cytotoxic activity of low molecular weight polyphenols against human oral tumor cell lines. Anticancer Res *20:* 2825-2536, 2000.

7 Satoh K, Ida Y, Ishihara M and Sakagami H: Interaction between sodium ascorbate and polyphenols. Anticancer Res *19:* 4177-4786, 1999.

8 Terasawa K, Hosoya H, Sugita Y, Yokoe I and Sakagami H: Effects of anticancer drugs, metals and antioxidants on cytotoxic activity of benzothiepins/benzoxepins. Anticancer Res *20:* 2951-2954, 2000.

9 Ohara-Imaizumi M and Kumakura K: Effect of imidazole compounds on catecholamine release in adrenal chromaffin cells. Cell Mol Neurobiol *12:* 273-283, 1992.

10 Musgrave IF, Krautwurst D, Hescheller J and Schulz G: Clonidine and cirazoline inhibit activation of nicotinic channels in PC-12 cell. Ann NY Acad Sci *763:* 272-283, 1995.

11 Lysko PG, Cox JA, Vigano A and Henneberry RC: Excitory amino acid neurotoxicity at the N-methyl-D-aspartate receptor in cultured neurons' pharmacological characterization. Brain Res *499:* 258-266, 1989.

12 Olmos G, Alemany R, Boronat MA and Garcia-Sevilla JA. Pharmacologic and molecular discrimination of 12-imidazoline receptor subtypes. Ann NY Acad Sci *21:* 144-160, 1999.

13 Milhaud D, Fagni L, Bockaert J and Lafon-Cazal M: Imidazoline-induced neuroprotective effects result from blockade of NMDA receptor channels in neuronal cultures. Neuropharmacol *39:* 2244-2254, 2000.

14 Olmos G, Degregorio-Rocasolano N, Regalado MP, Gesull T, Boronat MA, Trullas R, Villarroel A, Lerma J and Garcia-Sevilla JA. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br J Pharmacol *127:* 1317-1326, 1999.

15 Sugita Y, Yin S and Yokoe I: Reaction of 3-iodochromone with nucleophiles 2. Reaction with mercaptoazoles. Heterocycles *53:* 2191-2199, 2000.

16 Terasaka H, Takayama F, Satoh K, Fujisawa S and Sakagami H: Effect of antioxidants on radical intensity and cytotoxicity of hydroquinone. Anticancer Res *20:* 3357-3362, 2000.

17 Sakagami H and Satoh K: Prooxidant action of two antioxidants: Ascorbic acid and gallic acid. Anticancer Res *17:* 221-224, 1997.

18 Yanagisawa-Shiota F, Sakagami H, Kuribayashi N, Iida M, Sakagami T and Takeda M: Endonuclease activity and induction of DNA fragmentation in human myelogenous leukemic cell lines. Anticancer Res *15:* 259-266, 1995.

19 Sakagami T, Satoh K, Ishihara M, Sakagami H, Takeda F, Kochi M and Takeda M: Effect of cobalt ion on radical intensity and cytotoxic activity of antioxidants. Anticancer Res *20:* 3143-3150, 2000.

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